Fig. 1. Assay of serum creatine kinase

Reye's syndrome apparently represents another form of pathology in which a similar inactivation occurs. The kinetic properties of the reactivation of CK from a Reye's syndrome patient are analyzed to provide an explanation for the origin of the inhibition and a possible biochemical link between the two pathologies.

Creatine kinase normally has an activation lag phase of about 3 min in the presence of the reagents used in its analytical determination. Pre-incubation for the duration of this lag phase is followed by the appearance of a linear initial velocity, allowing assay of the enzyme activity (Figure 1). The essential activating substance in the reagent mixture can be one of several sulfhydryl agents (e.g., dithiothreitol, β-mercaptoethanol, cysteine, or glutathione) (5).

Figure 1 shows assay of increased CK for serum from a patient with myocardial infarction (control, A), from an acutely ill Reye's syndrome patient (B), and an equal mixture of the two sera (C). Several conclusions can be derived directly by inspection of Figure 1:

(a) The identical reactivity of the mixed serum assay, C, with the control assay, A, indicates that no component of the Reye's syndrome patient's serum is interfering in the assay system. This experiment also precludes the presence of a free, circulating inhibitor for CK in Reye's syndrome.

(b) Regardless of changes in concentration (over more than a 10-fold range) of the sulfhydryl activation reagent (RSH) added to the Reye's syndrome patient's serum, we saw no change in the lag period of about 12 min (Figure 1, B).

For both sera, an activation reaction occurring directly, as shown in equation

\[
\text{CK}_{\text{inactive}} + \text{RSH} \rightarrow \text{CK}_{\text{active}}
\]

would have a lag period varying inversely with the concentration of RSH. The total absence of any such dependence, over a wide range of concentrations, requires the postulation of a pre-equilibrium binding step as in enzyme kinetics, with inactive enzyme saturated by sulfhydryl reagent:

\[
\text{CK}_{\text{inactive}} + \text{RSH} \rightarrow \text{CK}_{\text{active}} \cdot \text{RSH}
\]

The actual activation step, designated by \( k \), is rate limiting. The occurrence of unique lag periods (i.e., unique rate constants \( k \), for activation), each unaffected by \( \text{RSH} \) concentration, indicates that \( \text{CK}_{\text{inactive}} \) in the usual clinical condition (myocardial infarction) is of a different molecular form from \( \text{CK}_{\text{inactive}} \) in Reye's syndrome or metastatic carcinoma. Because the isoenzyme behavior is similar in these two conditions, the molecular difference most likely results from covalent attachment to CK of some metabolite that is abnormally present in the serum. The necessity for removal of this attached metabolite from the essential sulfhydryls of the enzyme, as indicated schematically in the inset in Figure 1, is the likely role of sulfhydryl activating agents and the origin of the increased incubation period required.

Severe metabolic anomalies are evident in Reye's syndrome (6, 7), many of which are related to greatly augmented gluconeogenesis. The cancer patient with metastases to the liver is in a similar metabolically compromised state. Perhaps a common biochemical link in the two disease states is being reflected by CK inhibition.

The purpose of this communication is to alert investigators and clinicians to the possibility of an inhibited CK in Reye's syndrome. In view of the important prognostic significance of CK activity in this disease, normal or low values should be viewed cautiously in Reye's syndrome patients until the inhibitory phenomena are better understood.

References

Donald T. Forman
Helen Kieffer
Samuel H. Grayson
Evanston Hospital and Northwestern Univ. Med. School
Evanston, Ill 60201

Reminder of an Earlier Report

To the Editor:

Recently Clark et al. (1) reported on the time dependency of inhibition of human liver lactate dehydrogenase (EC 1.1.1.27; LD) by 1 mol/liter urea, and indicated that this phenomenon had not been previously described. Ten years ago I described the time dependency of inhibition of human liver LD and further demonstrated that this inhibition was also dependent on lactate concentration (2). At high lactate concentrations, the inhibition by urea is significantly delayed and completely abolished above 0.9 mol/liter L(+)-lactate. High lactate concentrations, with or without added urea, preferentially inhibit human heart LD. The comparison of LD activity in the presence of L(+)-lactate (1 mol/liter) and L(+)-lactate (10 mmol/liter) plus urea (1 mol/liter) after a 10-min incubation at 37 °C in pH 8.2 tris(hydroxymethyl)methylamine formed the basis of a chemical differentiation of heart and liver LD (2).

References

Arthur L. Babson
Dept. of Diagnostics Research & Development
Warner-Lambert Research Institute
Morris Plains, N. J. 07950

Ed note: The Letter in question dealt with LD-5 in particular, rather than total LD activity.